

Potential Markers for the Discrimination of Active and Latent Tuberculosis Infection: Moving the Research Agenda Forward

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INTRODUCTION

Tuberculosis control relies on the identification and preventive treatment of individuals who are latently infected with Mycobacterium Tuberculosis (**MTB**) [1]. The term latent tuberculosis was introduced by Clemens von Pirquet, who developed the Tuberculin Skin Test (**TST**) in 1907 [2]. Since nearly a third of the world's population is estimated to have Latent M. Tuberculosis Infection (**LTBI**) [3], a significant improvement in the understanding of the MTB "latency" is necessary to be able to put forth the research priorities for control of Tuberculosis (**TB**) especially in developing counties [2,4]. The major drawback currently is that none of the diagnostic assays for LTBI are sufficiently sensitive or specific [2]. In the absence of a gold standard, the most widely used diagnostic test for LTBI remains the TST using Purified Protein Derivative (**PPD**). However, this test has several limitations, including false-negative results particularly in some high-risk groups, and false-positive results in subjects exposed to non-tuberculous mycobacteria and

BCG-vaccinated individuals [5]. In addition, two tests based on the *in vitro* release of Interferon Gamma (**IFN- γ**) by circulating lymphocytes (hence, Interferon Gamma Release Assays (**IGRAs**)) upon stimulation with the *M. tuberculosis*-specific antigens Early Secreted Antigenic Target, 6 kilodaltons (**ESAT-6**) and Culture Filtrate Protein 10 kda (**CFP-10**) (the T-SPOT.TB test-Oxford Immunotec, Oxford, United Kingdom and the Quanti FERON-TB Gold test-QFT-G, Cellestis, Victoria, Australia), have been introduced for the diagnosis of MTB infection [6]. Although TST and IGRAs can help to distinguish MTB-infected from uninfected patients, they still cannot distinguish between active and latent TB cases [7].

Several methods have been suggested as tools to discriminate active TB from LTBI (Table 1). Recently, differences in the levels of microRNAs between patients with active TB and individuals with LTBI have been reported [8-10]. In Miotto et al. study, the 134 miRNAs showed relevant differences in serum level of LTBI and active pulmonary TB subjects [11].

Table 1: Probable markers for discrimination of active TB and LTBI.

Methods			References
Levels of microRNAs			[8-11,19]
Flow cytometry technique or Intracellular Cytokine Staining (ICC)			[12,56,57]
Gene expression of innate immunity genes	Antimicrobial molecules	DEFA1,DEFA4, LTF, CD64, BPI, and FPR1	[20]
	Members of the Ras-associated small GTPase (Rab) family	Rab13, Rab24, and Rab33A	[15]
	Soluble Markers of the Toll-Like Receptor 4 Pathway	Soluble (s) CD14 and Myeloid Differentiation-2 (MD-2), Lipopolysaccharide (LPS)	[34]
	TLR6		[16]
	ATP10A, FLNB		[16]
	IL-1RA, IL-2RA, IL-1 β		[16,45,46,51,58]
	LTF, guanylate binding protein 5 and Granzyme A)		[18]
	FCGR1B, CD64		[(7,18]
	Chemokine	CCL	[19]
		CXCL	[16,19,35]
	C1q components, C1qA, C1qB, and C1qC		[17,18,52]
	Level of host heat shock proteins (Hsps)	Host Hsp25, Hsp60, Hsp70, and Hsp90 and MTBHsp16	[53]
Cell surface proteins associated with particular states of the memory response on MTB antigen-specific CD4 T cells	CD27		[7,22]
	CD69		[23]
	CD45RA		[24]
	CCR7		[24]
	CD127		[7,21]
	Heparin-Binding Hemagglutinin (HBHA)		[7,26-29]
	Granzyme A serum levels		[32]
Serological methods	Plasma level of Heme Oxygenase-1 (HO-1)		[31]

	C-Reactive Protein (CRP)		[31]
	Serum Amyloid Protein-A (SAA)		[31]
	Lipolytic enzymes	(LipY, Rv0183, Rv1984c and Rv3452)	[31]
		(LipY, Rv0183, Rv1984c and Rv3452)	[33]
Antibody responses to MTB antigens	Lipoarabinomannan, anti-trehalose 6,6'-dimycolate, and tubercular-glycolipid antigen		[55]
	α -Crystallin (ACR)		[54,55]
	Ferredoxin A (FdxA)		[54]
	Alanine Dehydrogenase (AlaDH)		[54]
	38-kDa Ag		[54]
Cytokine measurements	IL-1a		[45,46]
	IL-1b		[46]
	IL-2		[37-41,45,46,58-61]
	IL-4		[45,46,48,58]
	IL-5		[45,46,48,58]
	IL-6		[45,46,48,58]
	IL-7		[37,45,46,48,58]
	IL-8		[45,46,58]
	IL-10		[37,45,46]
	IL-12 (p40)		[42,45,46,48,51,58]
	IL-13		[35,38,42,45,46,48,58]
	IL-15		[45,46,58]
	IL-17		[31,35,38,42,45,46,48,58]
	IL-18		[42]
	IP-10		[37,45,46,48,51,58,59,62]
	IFN- α		[45,48,51,58]
	Tumor Necrosis Factor Alpha (TNF-α)		[31,35,37,38,42,45,46,48,50,51,58,59]
	Transforming Growth Factor- α (TGF-α)		[46,51]
	Monocyte Chemotactic Protein 1 (MCP-1)		[37,45,46,58]
	MCP-2		[62]
	Macrophage Inflammatory Protein (MIP) 1a		[45,46,58]
	MIP-1b		[37,45,46,51,58]
	sCD40L		[46,51]
	Vascular Endothelial Growth Factor (VEGF)		[46,49,51]
	GM-CSF		[45,48,58]
	MIG		[45,58]
	Eotaxin		[45,58]
	RANTES		[45,58]
	Epidermal Growth Factor (EGF)		[51]

Another method is the flow cytometry/Intracellular Cytokine Staining (**ICS**) [12]. Recently, researchers have used ICS to try and discriminate active from latent TB by differences in the combinations of cytokines produced by M. tuberculosis antigen-specific CD4 T cells [13,14].

Another approach has been to measure gene expression of innate immunity genes such as antimicrobial molecules, members of the Ras-associated small GTPase (Rab) family [15], CXCL10, ATP10A, TLR6, IL2RA, and FLNB [16] and a unique subset of genes (LTF, guanylate binding protein 5, FCGR1B, CD64 and Granzyme A) [17,18].

In Lu et al. study, five genes (CXCL10, ATP10A, TLR6, IL2RA, and FLNB) were differentially expressed between TB and LTBI group and the fold changes in TB patients were greater than LTBI individuals [16]. Yu et al. reported that the level CXCL11 and CXCL12 were significantly higher in the LTBI group [19]. Moreover, Alpha-Defensins 1 and 4 (**DEFA1 and DEFA4**) DEFA1, DEFA4, Lactoferrin (**LTF**), Cluster of Differentiation 64 (**CD64**), bactericidal permeability increasing protein (BPI), and Formyl Peptide Receptor 1 (**FPR1**) were differentially expressed between TB patients and LTBI group [20].

Maertzdorf et al. identified a unique subset of genes (FCGR1B, CD64, LTF, guanylate binding protein 5 and Granzyme A) have capable of distinguishing active TB from LTBI [18]. In Jacobsen et al. study, RNA of Rab molecules was differentially expressed between PBMC from TB patients and healthy MTB infected contacts [15].

Another approach used for discriminating actively from latently infected individuals is the measurement of CD4 T cell memory markers on MTB antigen-specific cells [21]. Markers that have been suggested include CD27 [22], CD69 [23], CD45RA and CCR7 [24], and CD127 [7,21]. It has been reported that subjects with active TB weakly expressing CD27 than those with LTBI [7,22,25].

Another possible avenue of discrimination has been the identification of particular MTB antigens that appear to have stronger T cell responses in the latent population such as Heparin-Binding Haemagglutinin (**HBHA**) [7,26,27]. HBHA has been found by IGRAs to induce significantly stronger T cell responses in latently infected individuals than in individuals with active disease [7,27-29]. However, one of the current challenges is that HBHA is a methylated antigen and that the methylation is important for the HBHA-specific T-cell responses [30].

Serologic method such as measurement of plasma Heme Oxygenase-1 (**HO-1**) [31], Granzyme A serum levels [32], cytokine and acute phase protein levels, chemokine, lipolytic enzymes [31,33] and Serum Amyloid Protein-A (**SAA**) [31] have been reported as a discriminating tool among actively from latently infected individuals.

Plasma HO-1 levels can be used to distinguish active TB from LTBI with remarkably high accuracy [31]. Significant increased levels of HO-1, CRP and SAA were detected in plasma from patients with active TB compared to individuals with LTBI [31].

Granzyme A serum levels in TB patients were significantly lower than values found in LTBI subjects. Using a cut-off value of granzyme A of <3.425 pg/ml resulted the sensitivity and the specificity of 29.41% and 94.74% in these two groups respectively [32].

Plasma level of soluble markers of the Toll-Like-Receptor 4 (**TLR-4**) pathway is considered as another possible approach of discrimination [34]. It has been reported that levels of LPS, MD-2 and sCD14 can discriminate between active TB and LTBI [34].

In Feruglio et al. study, plasma levels of MD-2 and sCD14 were significantly higher in the active TB group compared to the LTBI group. In addition, plasma levels of LPS were higher, although not significant; in the active TB group compared to the LTBI group [34].

Brust et al. indicated that the lipolytic enzymes tested are allowing to distinguish active TB from LTBI [33].

Several studies have indicated that cytokine measurements might discriminate between active TB and LTBI [19,24,35-47]. However, inconsistency in cytokine results was observed. The reason for the variability of the studies is probably due to the many variations such as the stimulation assays used, the use of whole blood versus density gradient-separated mononuclear cells, the duration of antigen stimulation, the use of costimulatory antibodies, and the antibody-fluorophore combinations [7].

According to the meta- analysis, IL-2 is the valid marker for discrimination of active and latent TB with the pooled sensitivity and specificity of 81% and 95%, respectively [40]. Cytokines interleukin-2 and Transforming Growth Factor-Beta 1 (TGF- β 1) were shown to distinguish between stages of MTB infection after blood was stimulated with the QFT peptides [46]. In Yu et al. study [19], the level of cytokine interleukin-2 and the chemokines IP-10 were significantly higher in the LTB group.

In Borgström et al. study, IP-10 levels were significantly higher in patients with active TB than in LTBI [48]. Whittaker et al. [43] reported that the baseline levels of IP-10 were increased in active TB and in LTBI, but there is no significant difference of stimulated levels of IP-10 between active TB and LTBI. According to Amanatidou et al. report [44], IP-10 had 83.3% sensitivity, 79.6% specificity, 80.4% positive predictive value and 82.7% negative predictive value for the diagnosis of LTBI in young children, while in Whittaker et al. study, this marker did not distinguish between active TB and LTBI in children [43].

In Frahm et al. study, expression levels of IL-15 and MCP-1 were found to be significantly higher in the active TB subjects compared to LTBI group [45].

Chegou et al. reported that measurement of the levels of IFN- α 2, IL-1Ra, sCD40L, IP-10 and VEGF in QFT-IT supernatants may be a useful method for diagnosing TB disease and differentiating between active TB disease and MTB infection in children [49].

According to Hur et al. report, IL-17 and IL-10 production might differentiate individuals with LTBI from active TB [35]. In Wang et al. study TNF- α could distinguish between active and LTBI [50]. Sutherland et al. found that TNF- α production following ESAT-6/CFP-10 stimulation and TNF- α and IL-12(p40) following TB10.4 stimulation were significantly higher from TB cases compared to LTBI group [42],

Chegou et al. reported Epidermal Growth Factor (**EGF**) soluble CD40 Ligand (**sCD40L**), antigen stimulated levels of EGF, and the background corrected antigen stimulated levels of EGF and Macrophage Inflammatory Protein (**MIP**)-1 β as the most informative single markers for differentiation between TB disease and LTBI, with AUCs of 0.88, 0.84, 0.87, 0.90 and 0.79, respectively. In addition, EGF, VEGF, TGF- α and sCD40L levels were higher in TB patients and combinations between EGF, sCD40L, VEGF, TGF- α and IL-1 α could differentiate between TB infection states [51].

Recently, comparing whole blood microarray data from LTBI and TB patients C1q mRNA showed that compared to LTBI, TB patients displayed significantly increased C1q mRNA expression in peripheral blood cells [17,18,52]. The ROC analysis revealed that C1qC mRNA expression alone in PBMC can distinguish active TB from LTBI with a sensitivity of 74% and specificity of 82%, respectively [17]. Maertzdorf et al. reported an increase in C1qA and C1qB, but not C1qC, in patients with active TB [18].

Another approach used for discriminating actively from latently infected individuals is measurement of the levels of the heat shock proteins Hsp(s). Shekhawat et al. reported Hsp25, Hsp60, Hsp70 and Hsp90 and MTB Hsp16 were significantly elevated in the active TB group compared to the high-risk exposure group, the low-risk exposure group and the control group [53].

Since antibody profiles associated with LTBI differed from those associated with active TB, another approach of discrimination might be antibody response during latent infection and active disease [54]. However the production of specific antibodies depends on several factors, such as the antigen's amount, accessibility, chemical structure, and its localization [55].

In Davidow et al. study, antibodies against AlaDH, the 38-kDa Ag, ESAT-6, and the 16-kDa Ag distinguished active TB from inactive TB [54].

Senoputra et al. reported that TB patients had significantly higher plasma levels of anti-tubercular-glycolipid antigen, anti- α -crystallin and anti- trehalose 6,6'-dimycolate compared to LTBI groups [55].

Finally, several markers have potential to support the discrimination between TB disease and LTBI. Since the TST and IGRAs cannot distinguish active TB from LTBI, these markers could be useful in differentiating these two stages of TB.

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